Selective implications of ATM and ATR kinases in hippocampal neurons

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Introduction

Ataxia Telangiectasia Mutated (**ATM**) and **Ataxia Telangiectasia and Rad3-related** (**ATR**) are **serine/threonine protein kinases**, belonging to the phosphatidylinositol 3-kinase-related kinases (PIKKs) family.
They are mostly known for their role at the peak of the signalling cascade mediating **DNA damage repair**, respectively upon double- and single-strand breaks. Furthermore, **ATM** and **ATR** are also localized in the cytoplasm where they exert DDR-unrelated functions.
Particularly, in neurons they participate in the **control of synaptic vesicles trafficking** and in mechanisms for **neurotransmitter release. ATM** interacts with β-adaptin and its neuronal-specific homolog β-NAP, that is required for synaptic vesicle formation. Moreover, **ATM** and **ATR** respectively phosphorylate VAMP2 and synapsin-I and in cortical neurons, **ATM** associates exclusively with excitatory vGLUT+ vesicles, while **ATR** only with inhibitory vGAT+ vesicles, thus their correct expression is essential to maintain E/I balance.
Lastly, in our lab, we already demonstrated that **reduced levels of ATM in hippocampal neurons** cause an **imbalance in the E/I ratio towards inhibition**, determined by an early GABA switch and increased KCC2 expression.

Aim

To investigate how and at which extent **ATM** and **ATR kinases** regulate the **correct establishment of synaptic plasticity** in hippocampal neurons by treating developing and mature cultures with selective ATM or ATR kinase activity inhibitors

Background - 1

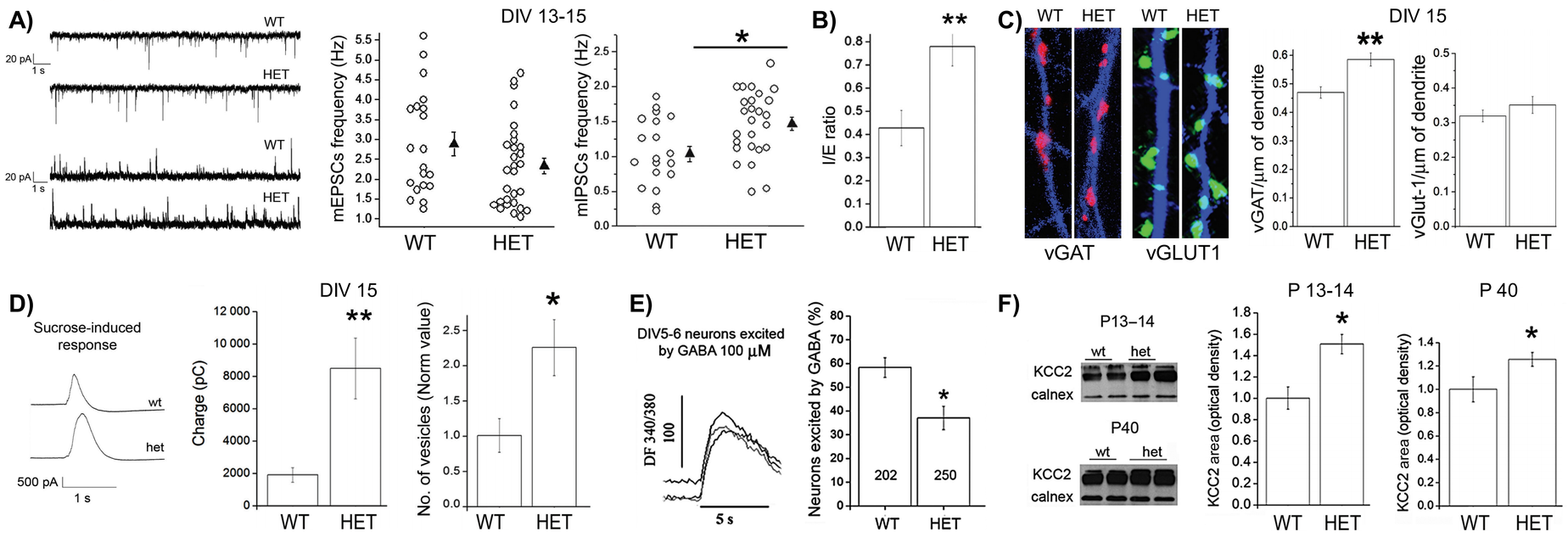


Figure 1. Enhanced inhibitory transmission in mouse hippocampal ATM$^{+/-}$ neurons associates to an early GABA switch and increased KCC2 expression

(A) ATM HET neurons at DIV 13-15 show increased mIPSCs frequency compared to WT
(B) I/E ratio indicates an enhanced inhibitory tone in HET cultured neurons
(C) Increased vGAT-positive puncta (red) per unit length of dendrite (β–tubulin-positive filament; blue) indicate increased inhibitory synapse density in HET neurons
(D) Upon hypertonic sucrose solution delivery, an increased charge is transferred at the inhibitory synapse in HET neurons, consistently with a higher number of inhibitory synaptic vesicles
(E) Ca^{2+} imaging at DIV 5-6 shows a smaller percentage of HET neurons responding to acute GABA administration (100 µM) compared to WT, indicating an early GABA switch
(F) KCC2 is augmented in hippocampal tissues from P14 and P40 HET mice

*Unpaired t test, *p<0.05, **p<0.01*

Pizzamiglio et al. 2016, Cerebral Cortex

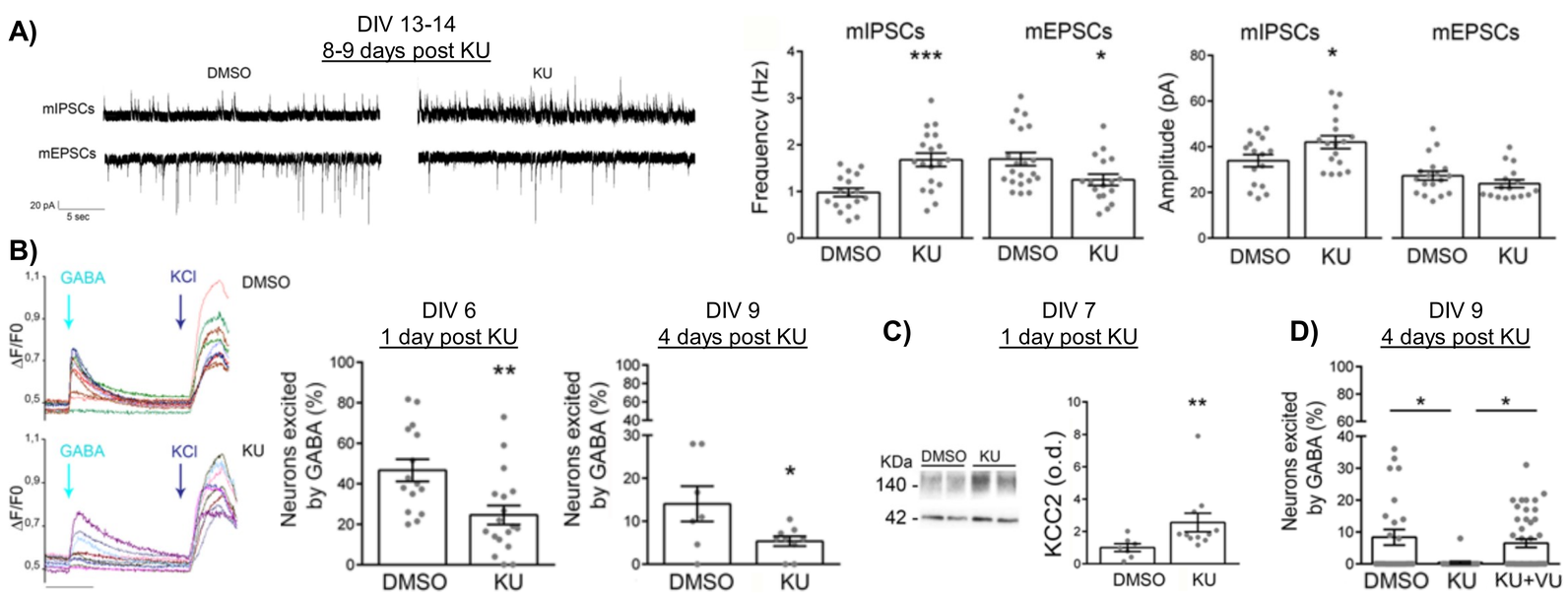
Background - 2

Figure 2. ATM kinase activity inhibitor KU55933 regulates GABA development by boosting KCC2 protein levels in mouse hippocampal neurons

(A) DIV 13-14 neurons treated with KU 1 µM at DIV 5 show increased mIPSCs frequency and amplitude and decreased mEPSCs frequency
(B) Ca^{2+} imaging of neurons treated with KU at DIV 5 and analysed after 1 or 4 days shows a smaller percentage of neurons responding to acute GABA administration (100 µM) compared to DMSO-treated neurons
(C) KCC2 signal is augmented in hippocampal neurons at DIV 7 after 1 day of treatment with KU
(D) Ca^{2+} imaging shows no differences in the percentage of GABA-responding neurons in cultures treated with both KU and the KCC2 blocker VU 1 µM compared to DMSO-treated neurons, indicating that the defects previously detected are mediated by the higher KCC2 expression

*(A, B) Unpaired t test; (A mEPSCs amplitude, C) Mann-Whitney U test ; (D) Kruskal-Wallis test followed by Dunn's multiple comparisons test; *p<0.05, **p<0.001*

Pizzamiglio et al. 2021, JCI insight



Results - 1

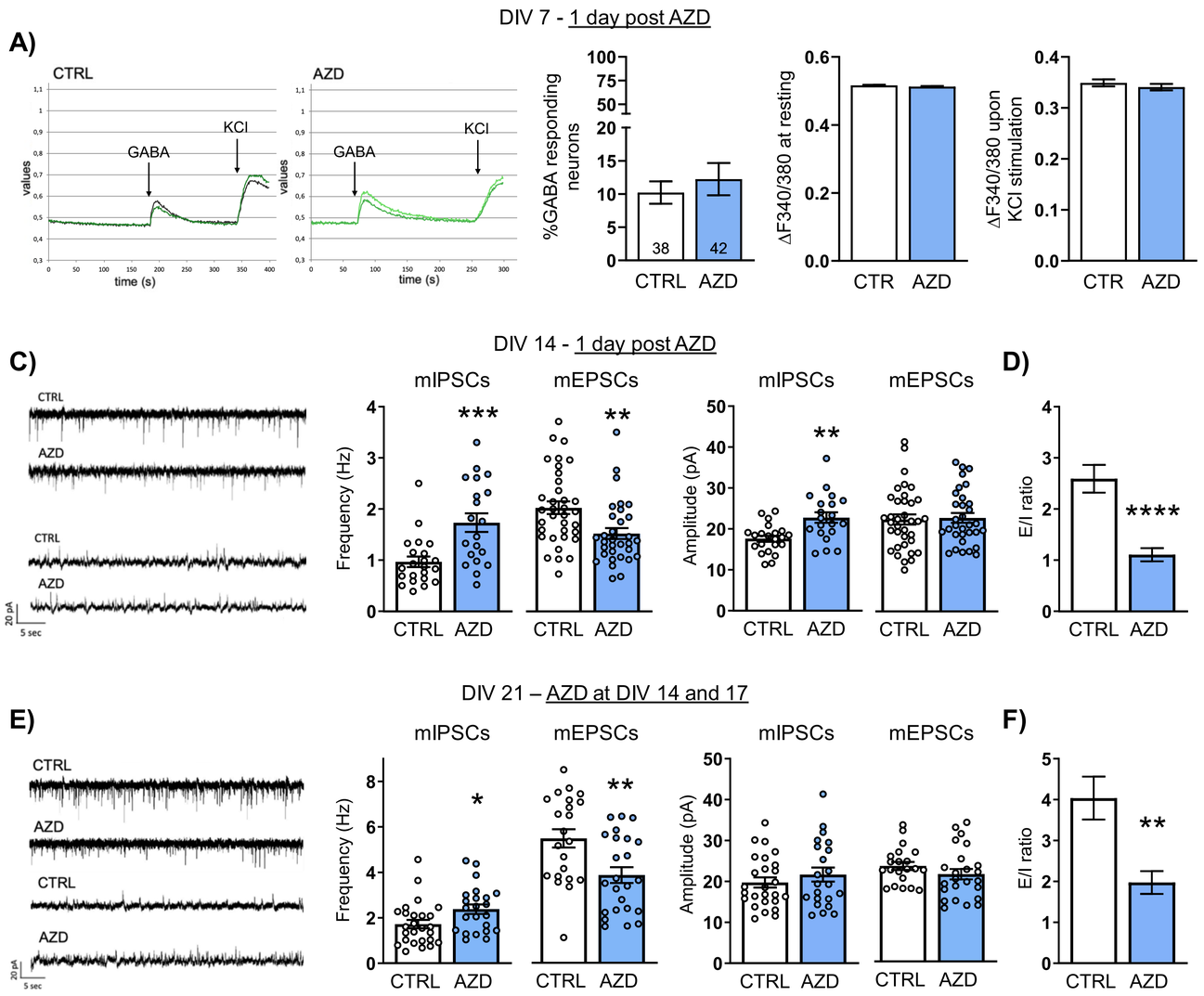


Figure 3. ATR kinase activity inhibitor AZD enhances the inhibitory tone of mature hippocampal cultured neurons

(A) Neurons treated with AZD at DIV 6 and analysed 1 day after show no differences in terms of percentage of GABA-responders and Ca^{2+} concentration at resting state and upon KCl stimulation
(B) Mature neurons treated acutely with AZD show increased mIPSCs frequency and amplitude and decreased mEPSCs frequency
(D) Mature neurons treated chronically with AZD show increased mIPSCs frequency and decreased mEPSCs frequency
(C-E) E/I ratios indicate an enhanced inhibitory tone in mature neurons treated acutely or chronically with AZD

*(A, B and D amplitudes, C, E) Mann-Whitney U test ; (D, B and D frequencies) Unpaired t test ; *p<0.05, **p<0.001*

Results - 2

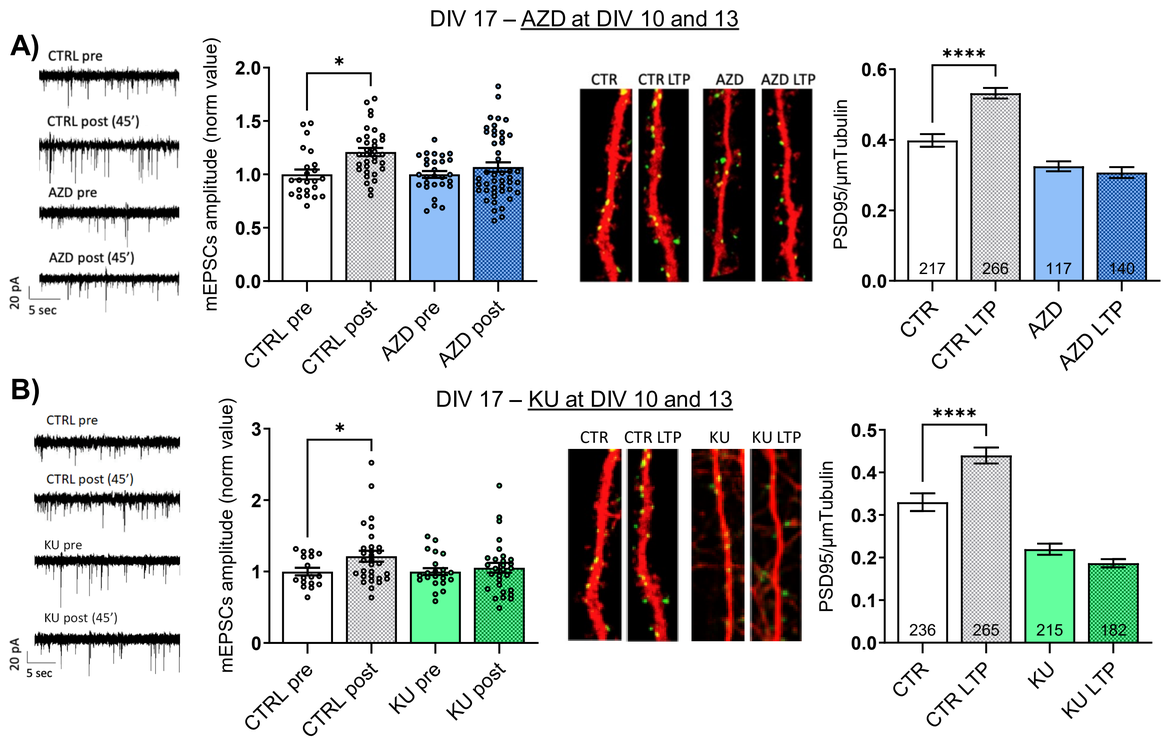


Figure 4. Chronic AZD and KU55933 treatment impair long term potentiation (LTP) in mature hippocampal neurons

Mature neurons treated chronically with AZD (A) or KU (B) show no differences in mEPSCs amplitude and in the density of PSD95-positive puncta (green) per unit length of dendrite (β–tubulin-positive filament; red) upon LTP induction indicating impaired LTP

*Kruskal-Wallis test followed by Dunn's multiple comparisons test; *p<0.05, **p<0.001*

Conclusions

ATM and **ATR** display **partially overlapping functions** in cultured **hippocampal neurons**. As a matter of fact, in mature neurons, the inhibition of either **ATM** or **ATR** impairs the **normal induction of plasticity processes**, but only **ATM kinase** controls the proper development of **GABA switch** in early postnatal hippocampal cultures.
In the future, therefore, we will investigate how and at which extent **ATM** and **ATR** are involved in **neurodevelopmental** and **neurodegenerative diseases** in which these processes are known to be impaired.

References

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